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A NOVEL METHOD OF DIAGNOSING, MONITORING, STAGING AND TREATING GYNECOLOGICAL AND PROSTATIC CANCERS

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating cancers, particularly gynecologic cancers including uterine, endometrial, breast and ovarian cancer, and prostate cancer.

BACKGROUND OF THE INVENTION

In women, gynecologic cancers account for more than one-fourth of the malignancies.

For example, endometrial cancer occurs at a rate of approximately 44,500 new cases per year with approximately 10,000 deaths per year. If diagnosed and treated early, when the cancer is still confined to the endometrium, cure can be achieved in approximately 95% of the cases by hysterectomy. Pap smears can show endometrial cancers but are effective in only 50% of the cases. For the remainder, abnormal vaginal bleeding is typically the first clinical sign of endometrial cancer.

Sarcoma of the uterus, a very rare kind of cancer in women, is a disease in which cancer (malignant) cells start growing in the muscles or other supporting tissues of the uterus. Sarcoma of the uterus is different from cancer of the endometrium, a disease in which cancer cells start growing in the lining of the uterus. Women who have received therapy with high-dose x-rays (external beam radiation therapy) to their pelvis are at a higher risk to develop sarcoma of the uterus. These x-rays are sometimes given to women to stop bleeding from the uterus. Like most cancers, sarcoma of the

uterus is best treated when it is found (diagnosed) early. Sarcoma of the uterus usually begins after menopause. When a patient has signs of such cancer, an internal pelvic examination is usually performed to detect any lumps or changes in shape of the pelvic organs. A Pap test may also be performed, however because sarcoma of the uterus begins inside the organ, this cancer is not usually detected by the Pap test. A dilation and curettage (D&C) may also be performed and a biopsy sample taken and examined microscopically.

It is estimated that one of every nine women in America will develop breast cancer sometime during her life based on a lifespan of 85 years. Annually, over 180,000 women in the United States are diagnosed with breast cancer and approximately 46,000 die from this disease. Every woman is at risk for breast cancer. However, a woman's chances of developing breast cancer increase as she grows older; 80 percent of all cancers are found in women over the age of 50. There are also several risk factors that can increase a woman's chances of developing breast cancer. These include a family history of breast cancer, having no children or the first child after the age of 30, and an early start of menstruation. However, more than 70 percent of women who develop breast cancer have no known risk factors. Less than 10 percent of breast cancer cases are thought to be related to the BRCA1 gene discovered in 1994. Researchers are now investigating the role of other factors such as nutrition, alcohol, exercise, smoking, and oral contraceptives in development of this gynecologic cancer. Mammograms, or special x-rays of the breast, can detect more than 90 percent of all cancers.

Carcinoma of the ovary is another very common gynecologic cancer. In fact, ovarian cancer causes more deaths than any other cancer of the female reproductive system. Approximately one in 70 women develop ovarian cancer

during their lifetime. An estimated 14,500 deaths in 1995 resulted from ovarian cancer. Ovarian cancer often does not cause any noticeable symptoms. Possible warning signals include an enlarged abdomen due to an accumulation of fluid or vague digestive disturbances (discomfort, gas or distention) in women over 40. In rare cases abnormal vaginal bleeding also occurs. Pap tests do not detect ovarian cancer. Thus, periodic, complete pelvic examinations are important and recommended annually for women over 40.

In men, cancer of the prostate is the most prevalent malignancy, excluding skin cancer, and is an increasingly prevalent health problem in the United States. In 1996, it was estimated that in the United States, 41,400 deaths would result from this disease, indicating that prostate cancer is second only to lung cancer as the most common cause of death in the same population. Treatment decisions for an individual are linked to the stage of prostate cancer present in that individual. A common classification of the spread of prostate cancer was developed by the American Urological Association (AUA). The AUA classification divides prostate tumors into four stages, A to D. Stage A, microscopic cancer within prostate, is further subdivided into stages A1 and A2. Sub-stage A1 is a well-differentiated cancer confined to one site within the prostate. Treatment is generally observation, radical prostatectomy, or radiation. Sub-stage A2 is a moderately to poorly differentiated cancer at multiple sites within the prostate. Treatment is radical prostatectomy or radiation. Stage B, palpable lump within the prostate, is further subdivided into stages B1 and B2. In sub-stage B1, the cancer forms a small nodule in one lobe of the prostate. In sub-stage B2, the cancer forms large or multiple nodules, or occurs in both lobes of the prostate. Treatment for both sub-stages B1 and B2 is either radical prostatectomy or radiation. Stage C is a large cancer mass involving most or all of the prostate and is further subdivided into two stages.

In sub-stage C1, the cancer forms a continuous mass that may have extended beyond the prostate. In sub-stage C2, the cancer forms a continuous mass that invades the surrounding tissue. Treatment for both these sub-stages is radiation with or without drugs. The fourth stage is metastatic cancer and is also subdivided into two stages. In sub-stage D1, the cancer appears in the lymph nodes of the pelvis. In sub-stage D2, the cancer involves tissues beyond lymph nodes. Treatment for both these sub-stages is systemic drugs to address the cancer as well as pain.

In all of these cancers, chances of survival are much better if the cancer is diagnosed at an early stage. Further, treatment decisions for the individual are linked to the stage of the cancer present in that individual. However, current cancer staging methods are limited and some such cancers initially staged as not metastatic are actually metastatic. For example, as many as 50% of the cases of prostate cancer initially staged as A2, B, or C are actually stage D, metastatic. Discovery of metastasis is significant because patients with metastatic cancers have a poorer prognosis and require significantly different therapy than those with localized cancers.

Accordingly, there is a great need for sensitive and accurate methods for early detection and staging of gynecologic cancers such as endometrial, breast, uterine and ovarian cancer and prostate cancer in humans.

Steroid binding proteins, including uteroglobin and CC10, are a class of proteins which bind steroids along with methylsulfonyl metabolites of polychlorinated biphenyls. The exact function of members of this class of protein is uncertain. However, uteroglobin has been shown to inhibit PLA_2 mediated responses.

Gene and gene products homologous to uteroglobin are described in WO 97/34997 entitled Human Endometrial Specific Steroid Binding Factors I, II and III. The genes and their

encoded products are referred to as Human Endometrial Specific Steroid-Binding Factors I, II and III (hESF I, II, and III). Methods for utilizing these genes and gene products in research and diagnostic and clinical arts are disclosed. In particular, methods for detecting mutations in the hESFI, II or III gene or altered protein expression resulting from a mutant gene are indicated to be useful in diagnosing susceptibility to asthma and endometrial cancer.

A novel member of the uteroglobin family which is very similar to hESF II, referred to as BU101, is also described in WO 98/07857. BU101 is disclosed to be over-expressed in a percentage of breast tumors. Therefore, BU101 is suggested to be useful for the detecting, diagnosing staging, monitoring, prognosticating, preventing, treating and determining the predisposition of an individual to diseases and conditions of the breast such as breast cancer.

In WO 98/56248 methods for diagnosing breast cancer, endometrial cancer, endometriosis and endometrial fibroids via detection of a gene or gene product referred to therein as ESBPII and identical to BU101 are disclosed.

It has now been found that detection of ESBPII is also useful in diagnosing, monitoring, staging, prognosticating, imaging and treating uterine, ovarian and prostate cancer.

Accordingly, in the present invention, methods are provided for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating prostate cancer and gynecologic cancers including not only breast and endometrial cancer, but also uterine and ovarian cancer via ESBPII. ESBPII refers, among other things, to native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The amino acid sequence of a polypeptide encoded by SEQ ID NO:1 is depicted herein as SEQ ID NO:2. In the alternative, what is meant by the ESBPII as used herein, means the native mRNA encoded by the gene comprising the

polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of prostate cancer or gynecologic cancers by analyzing for changes in levels of ESBPII in cells, tissues or bodily fluids compared with levels of ESBPII in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein a change in levels of ESBPII in the patient versus the normal human control is associated with prostate cancer or a gynecologic cancer.

Further provided is a method of diagnosing metastatic prostate cancer or a metastatic gynecologic cancer in a patient which is not known to have metastasized by identifying a human patient suspected of having prostate cancer or a gynecologic cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for ESBPII; and comparing the ESBPII levels in such cells, tissues, or bodily fluid with levels of ESBPII in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in ESBPII levels in the patient versus the normal human control is associated with prostate cancer or a gynecologic cancer which has metastasized.

Also provided by the invention is a method of staging prostate cancer or a gynecologic cancer in a human by identifying a human patient having prostate cancer or a gynecologic cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for ESBPII; comparing ESBPII levels in such cells, tissues, or bodily fluid with levels of ESBPII in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in ESBPII levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of ESBPII is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring prostate cancer or a gynecologic cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for ESBPII; comparing the ESBPII levels in such cells, tissue, or bodily fluid with levels of ESBPII in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in ESBPII levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided is a method of monitoring the change in stage of prostate cancer or a gynecologic cancer in a human patient by monitoring levels of ESBPII in the patient. The method comprises identifying a human patient having prostate cancer or a gynecologic cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for ESBPII; comparing the ESBPII levels in such cells, tissue, or bodily fluid with levels of ESBPII in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in ESBPII levels in the patient versus the normal human control is associated with a cancer

which is progressing and a decrease in the levels of ESBPII is associated with a cancer which is regressing or in remission.

Further provided are antibodies which specifically bind ESBPII or fragments of such antibodies which can be used to detect or image localization of ESBPII in a patient for the purpose of detecting or diagnosing prostate cancer or a gynecologic cancer. Such antibodies can be polyclonal, monoclonal, or omniclonal or prepared by molecular biology techniques. The term "antibody", as used herein and throughout the instant specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be labeled with a variety of detectable labels including, but not limited to, radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in the treatment of diseases characterized by expression of a ESBPII. In therapeutic applications, the antibody can be used without or with derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging and prognosticating cancers by comparing levels of ESBPII with those of ESBPII in a normal human control. What is meant by levels of ESBPII as used herein, means levels of the native protein expressed by the gene comprising the polynucleotide sequence of SEQ ID NO:1. The polypeptide encoded by this polynucleotide sequence is depicted in SEQ ID NO:2. In the alternative, what is meant by levels of ESBPII as used herein, mean levels of the native mRNA encoded by the gene comprising the polynucleotide sequence of SEQ ID NO:1 or levels of the gene comprising the polynucleotide sequence of SEQ ID NO:1. Such levels are preferably measured in at least one of cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing overexpression of ESBPII protein compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including prostate cancer and gynecologic cancers such as uterine, endometrial, breast and ovarian cancer.

All the methods of the present invention may optionally include measuring levels of other cancer markers as well as ESBPII. Other cancer markers, in addition to ESBPII, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of prostate cancer or gynecologic cancers including breast, uterine, ovarian and endometrial cancer by analyzing for changes in levels of ESBPII in cells, tissues or bodily fluids of a human patient compared with levels of ESBPII in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in

levels of ESBPII in the patient versus the normal human control is associated with the presence of prostate cancer or a gynecologic cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues or bodily fluid levels of the cancer marker, such as ESBPII, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing the onset of metastasis in human patients with a gynecologic cancer or prostate cancer. In this method, a human cancer patient suspected of having a gynecologic cancer or prostate cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art.

In the present invention, determining the presence of ESBPII levels in cells, tissues or bodily fluid, is particularly useful for discriminating between prostate cancer or a gynecologic cancer which has not metastasized and prostate cancer or a gynecologic cancer which has metastasized. Existing techniques have difficulty discriminating between prostate cancer or gynecologic cancers which have metastasized and prostate cancer or gynecologic cancers which have not metastasized. However, proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker level measured in such cells, tissues or bodily fluid is ESBPII. Measured ESBPII levels in a human patient are compared with levels of ESBPII in preferably the same cells, tissue or bodily fluid type of a normal human control. That is, if the cancer marker being observed is ESBPII in serum, this level is preferably compared with the level of ESBPII in serum of

a normal human control. An increase in the ESBPII in the patient versus the normal human control is associated with prostate cancer or a gynecologic cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues or bodily fluid levels of a cancer marker, such as ESBPII, are at least two times higher, and most preferably are at least five times higher, than in preferably the same cells, tissues or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing or monitoring for metastasis, normal human control may preferably include samples from a human patient that is determined by reliable methods to have prostate cancer or a gynecologic cancer which has not metastasized.

Staging

The invention also provides a method of staging prostate cancer or gynecologic cancers in a human patient. The method comprises identifying a human patient having prostate cancer or a gynecologic cancer and analyzing cells, tissues or bodily fluid from such human patient for levels of ESBPII. The levels of ESBPII in the patient are then compared to levels of ESBPII in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in ESBPII levels in the human patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of ESBPII is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring prostate cancer or gynecologic cancers in a human patient having such

cancer for the onset of metastasis. The method comprises identifying a human patient having prostate cancer or a gynecologic cancer that is not known to have metastasized; periodically analyzing cells, tissues or bodily fluid from such human patient for ESBPII; comparing the ESBPII levels in such cells, tissues or bodily fluid with levels of ESBPII in preferably the same cells, tissues or bodily fluid type of a normal human control, wherein an increase in ESBPII levels in the human patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this invention is a method of monitoring the change in stage of prostate cancer or a gynecologic cancer in a human having such cancer. The method comprises identifying a human patient having prostate cancer or a gynecologic cancer; periodically analyzing cells, tissues or bodily fluid from such human patient for ESBPII; and comparing the ESBPII levels in such cells, tissues or bodily fluid with levels of ESBPII in preferably the same cells, tissues or bodily fluid type of a normal human control sample, wherein an increase in ESBPII levels in the human patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of ESBPII is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression (including protein levels), such as ESBPII of the present invention, in a sample derived from a patient are well known to those of skill in the art. Such assay methods include, without limitation, radioimmunoassays, reverse transcriptase PCR (RT-PCR) assays,

immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches: two-dimensional gel electrophoresis (2D electrophoresis) and non-gel based approaches such as mass spectrometry or protein interaction profiling. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to ESBPII, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to ESBPII. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent. Examples include, but are not limited to, horseradish peroxidase enzyme and alkaline phosphatase.

To carry out the ELISA, antibody specific to ESBPII is incubated on a solid support, e.g. a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time ESBPII binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to ESBPII and linked to a detectable reagent such as horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to ESBPII. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to ESBPII antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of ESBPII protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay can also be employed wherein antibodies specific to ESBPII are attached to a solid support and labeled ESBPII and a sample derived from the host are passed over the solid support. The amount of label detected which is attached to the solid support can be correlated to a quantity of ESBPII in the sample.

Nucleic acid methods can also be used to detect ESBPII mRNA as a marker for prostate cancer and gynecologic cancers. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e. gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the ESBPII gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the ESBPII gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but

not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels. First, proteins are separated by size using an electric current. The current acts uniformly on all proteins, so smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) obtained from the patient including tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum or any derivative of blood.

In Vivo Antibody Use

Antibodies against ESBPII can also be used *in vivo* in patients suspected of suffering from prostate cancer or gynecologic cancers such as ovarian, breast, endometrial and uterine cancer. Specifically, antibodies against a ESBPII can be injected into a patient suspected of having prostate cancer or a gynecologic cancer for diagnostic and/or therapeutic purposes. The use of antibodies for *in vivo* diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunoscintographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Lauffer, R.B. Magnetic Resonance in Medicine 1991 22:339-342). Antibodies directed against ESBPII can be used in a similar manner. Labeled antibodies against ESBPII can be injected into patients suspected of having a gynecologic cancer or prostate cancer for the purpose of diagnosing or staging of the disease status of the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadlinium (III) or Manganese (II) can be used in magnetic resonance imaging (MRI). Localization of the label permits determination of the spread of the cancer. The amount of label within an organ or tissue also allows determination of the presence or absence of cancer in that organ or tissue.

For patients diagnosed with prostate cancer or a gynecologic cancer, injection of an antibody against ESBPII can also have a therapeutic benefit. The antibody may exert its therapeutic effect alone. Alternatively, the antibody is conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its therapeutic effect. Drug monoclonal antibodies have been described in the art for example by Garnett and Baldwin, Cancer Research 1986 46:2407-2412. The use of toxins conjugated to monoclonal antibodies for the therapy of various cancers has also been described by Pastan et al. Cell 1986 47:641-648. Yttrium-90 labeled monoclonal antibodies have been described for maximization of dose delivered to the tumor while limiting toxicity to normal tissues (Goodwin and Meares Cancer Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of antibodies against ESBPII.

Antibodies which can be used in these *in vivo* methods include both polyclonal, monoclonal or omniclonal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art can also be used.

The present invention is further described by the following examples. These examples are provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

EXAMPLES

The examples are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out

as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'-3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control is used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ATP synthase 6 (ATPsy6), or 18S ribosomal RNA (rRNA) is used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample are used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene for every example was evaluated in normal and cancer tissue. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to the target gene. The results were analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels

of expression of the target gene in a particular tissue compared to the calibrator tissue.

The absolute numbers depicted in Table 1 are relative levels of expression of ESBPII in 12 normal different tissues. All the values are compared to normal lung (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 1: Relative Levels of ESBPII Expression in Pooled Samples

TISSUE	NORMAL
Brain	28
Heart	50
Kidney	374
Liver	3
Lung	1
Breast	10885
Prostate	590
Small Intestine	60
Spleen	1
Testis	308
Thymus	8
Uterus	16047

The relative levels of expression in Table 1 show that the highest level of expression of ESBPII mRNA is in uterus (16047) and the second highest levels of expression is in mammary gland (10885). Prostate (590), kidney (374), and testis (308) also express mRNA for ESBPII. These results established that ESBPII mRNA expression is highly specific for uterus and breast in female tissues, and prostate for male tissues.

The absolute numbers in Table 1 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 2.

The absolute numbers depicted in Table 2 are relative levels of expression of ESBPII in 76 pairs of matching

samples, ovarian cancer samples from 15 different individuals, and normal ovarian samples from 15 different individuals. All the values are compared to normal lung (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 2: Relative Levels of ESBPII Expression in Pooled Samples

Sample ID	Tissue	Cancer	Normal Adjacent Tissue	Normal
End10479	Endometrium 1	1189	143	
End8911	Endometrium 2	989	15772	
ENDO12XA	Endometrium 3	2226	6724	
ENDO28XA	Endometrium 4	14767	6701	
ENDO3AX	Endometrium 5	1284	2864	
ENDO5XA	Endometrium 6	20857	38388	
ENDO65RA	Endometrium 7	151825	1515	
ENDO8XA	Endometrium 8	115	4097	
End8963	Endometrium 9	149010	13308	
End4XA	Endometrium 10	274	12945	
End68X	Endometrium 11	751834	759691	
BLD32XK	Bladder 1	1	0	
BLD46XK	Bladder 2	26	21	
CLNAS45	Colon 1	1	1	
CLNRC01	Colon 2	0	2	
ClnB34	Colon 3	0	3	
CvxKS52	Cervix 1	0	17	
CvxNKS18	Cervix 2	0	20	
CvxNKs80	Cervix 3	0	778347	
Kid107XD	Kidney 1	1734	1239	
Kid106XD	Kidney 2	125	699	

Kid109XD	Kidney 3	274	637	
Kid10XD	Kidney 4	5814	1892	
Kid11XD	Kidney 5	423	537	
Kid124D	Kidney 6	100	332	
Kid12XD	Kidney 7	467	287	
Kid150D	Kidney 8	1862	134	
Kid373K	Kidney 9	388	330	
Kid5XD	Kidney 10	1239	1061	
Kid98XD	Kidney 11	100	391	
LIV15XA	Liver 1	3	2	
Liv94XA	Liver 2	39	0	
Lng60XL	Lung 1	0	2	
LngSQ81	Lung 2	0	12	
LNGC20X	Lung 3	5	26	
Mam47XP	Breast 1	1448	177	
Mam82XI	Breast 2	24	246	
MamA06X	Breast 3	10865	6047	
MamB011X	Breast 4	12915	1194	
Mam59X	Breast 5	648	114	
MamS079	Breast 6	24	33	
MamS123	Breast 7	81282	1598	
MamS516	Breast 8	5827	987	
MamS570	Breast 9	6125	15555	
PAN71XL	Pancreas 1	12	18	
Pan82XP	Pancreas 2	56	383	
Pan77X	Pancreas 3	0	0	
Pro18XB	Prostate 1	959	2798	
Pro20XB	Prostate 2	9642	2084	
Pro69XB	Prostate 3	2041	171	
Pro90XB	Prostate 4	251	3916	

	Pro65XB	Prostate 5	1296	832	
	Pro101XB	Prostate 6	2896	5753	
	Pro12B	Prostate 7	2750	111	
	Pro13XB	Prostate 8	33	551	
5	Pro23B	Prostate 9	310	309	
	Pro34B	Prostate 10	1261	1180	
	Pro78XB	Prostate 11	666	697	
	Pro84XB	Prostate 12	3214	972	
	Pro91X	Prostate 13	3835	989	
10	ProC215	Prostate 14	5312	nd	
	ProC234	Prostate 15	549	nd	
	ProC280	Prostate 16	873	nd	
	SmInt21XA	Small Intestine 1	9	12	
	SmInH89	Small Intestine 2	2	25	
15	StoAC44	Stomach 1	4	4	
	StoAC99	Stomach 2	3	2	
	Tst39X	Testis 1	29	146	
	UTR135XO	Uterus 1	2055	3616	
	UTR141XO	Uterus 2	69516	8024	
20	UTR85XU	Uterus 3	2947	7083	
	UTR23XU	Uterus 4	52501	6361	
	Ovr103X	Ovary 1	760	4	
	Ovr130X	Ovary 2	1	77	
	OvrG010	Ovary 3	1670	108	
25	OvrG021	Ovary 4	6	21	
	Ovr1005	Ovary Cancer 1	4716		
	Ovr1040	Ovary Cancer 2	2235		
	Ovr638A	Ovary Cancer 3	6		
	Ovr63A	Ovary Cancer 4	9		
30	Ovr1028	Ovary Cancer 5	8		

Ovr105O	Ovary Cancer 6	629		
Ovr1118	Ovary Cancer 7	2		
Ovr1157	Ovary Cancer 8	2694		
Ovr130X	Ovary Cancer 9	1587		
Ovr1461	Ovary Cancer 10	0		
Ovr180B	Ovary Cancer 11	3104		
Ovr371O	Ovary Cancer 12	803		
Ovr63A	Ovary Cancer 13	7		
OvrA1C	Ovary Cancer 14	4251		
OvrC360	Ovary Cancer 15	3		
Ovr18GA	Ovary Normal 1			4
Ovr2061	Ovary Normal 2			28
Ovr20GA	Ovary Normal 3			9
Ovr230A	Ovary Normal 4			28
Ovr233A	Ovary Normal 5			0
Ovr247A	Ovary Normal 6			113
Ovr25GA	Ovary Normal 7			88
Ovr32RA	Ovary Normal 8			47
Ovr638O	Ovary Normal 9			23
OvrC0004	Ovary Normal 10			16
OvrC179	Ovary Normal 11			8
Ovr35GA	Ovary Normal 12			18
Ovr40G	Ovary Normal 13			1
Ovr50GB	Ovary Normal 14			2
Ovr9RA	Ovary Normal 15			2

0= Negative
nd=Not Determined

In the analysis of matching samples, the higher levels of expression were in uterus, endometrium, breast, ovary, and prostate. There is also a lower expression of ESBPII in the kidney matching samples analyzed. The median expression in the kidney cancer samples (423), is one third the median

expression in the prostate cancer samples (1279). This pattern shows a high degree of specificity for female gynecologic tissues and prostate tissue. These results confirmed the tissue specificity results obtained with the panel of normal pooled samples (Table 1) for uterus, breast and prostate.

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 2 shows overexpression of ESBPII in 4 primary endometrial cancer tissues compared with their respective normal adjacent (endometrium samples #1, 4, 7, and 9). There was overexpression in the cancer tissue for 36.36% of the endometrial matching samples tested (total of 11 endometrium matching samples).

ESBPII is differentially expressed in the four matching samples for uterine cancer. Samples #1 and 3 show downregulation for the mRNA of ESBPII in cancer, whereas samples #2 and #4 show overexpression in the cancer samples. Of nine breast cancer matching samples analyzed, three showed underexpression of ESBPII (#2, 6, and 9) in cancer, whereas six had higher levels of ESBPII in cancer compared to the normal adjacent tissue (#1, 3, 4, 5, 7, and 8).

ESBPII is differentially expressed in the four matching samples for ovarian cancer. Samples #1 and 3 showed upregulation for the mRNA of ESBPII in cancer, whereas samples #2 and #4 showed overexpression in the normal adjacent tissue. Beside the four matching samples, thirty additional ovarian samples were analyzed. Fifteen cancer samples and 15 normal ovary tissue samples from different individuals. The median expression in the ovary cancer samples (629) is four times higher than the median expression in the normal ovary samples (16).

Altogether, the high level of tissue specificity for gynecological tissues, plus the mRNA differential expression in several of the primary uterus, endometrial, breast, and ovarian matching samples tested are indicative of ESBPII being a diagnostic marker for gynecologic cancers including uterine, endometrial, breast, and ovarian cancer. ESBPII is also differentially expressed in the 13 matching samples for prostate cancer. Samples #1, 4, 6, 8, and 11 showed downregulation of the mRNA of ESBPII in cancer, whereas samples #2, 3, 5, 7, 9, 10, 12, and 13 showed overexpression in the cancer tissue. The median expression in the prostate cancer samples (1279) is higher than the median expression in the normal prostate samples (972).

Altogether, the high level of tissue specificity for prostate tissue, plus the mRNA differential expression in several of the primary prostate matching samples tested are indicative of ESBPII being a diagnostic marker for prostate cancer.